Digestive Organ Sizes and Enzyme Activities of Refueling Western Sandpipers (*Calidris mauri*): Contrasting Effects of Season and Age

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ABSTRACT

We examined seasonal and age-related variation in digestive organ sizes and enzyme activities in female western sandpipers (*Calidris mauri*) refueling at a coastal stopover site in southern British Columbia. Adult sandpipers exhibited seasonal variation in pancreatic and intestinal enzyme activities but not in digestive system or organ sizes. Spring migrants had 22% higher total and 67% higher standardized pancreatic lipase activities but 37% lower total pancreatic amylase activity than fall migrants, which suggests that the spring diet was enriched with lipids but low in glycogen. Spring migrants also had 47% higher total intestinal maltase activity as well as 56% higher standardized maltase and 13% higher standardized aminopeptidase-N activities. Spring migrants had higher total enzymic capacity than fall migrants, due primarily to higher total lipase and maltase activities. During fall migration, the juvenile's digestive system was 10% larger than the adult's, and it was composed differently: juveniles had a 16% larger small intestine but a 27% smaller proventriculus. The juvenile's larger digestive system was associated with lower total enzymic capacity than the adult's due to 20% lower total chitinase and 23% lower total lipase activities. These results suggest that juvenile western sandpipers may process food differently from adults and/or have a lower-quality diet.

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Introduction

For birds, long-distance migration often is characterized by alternating periods of endurance flight and residence at stopover sites, where refueling occurs. Although fat is the primary fuel for these extended flights, some lean mass is also catabolized (Jenni and Jenni-Eirmann 1998), particularly from the digestive system (Karasov and Pinshow 1998; Battley et al. 2000). The digestive system serves two potentially conflicting functions during migration: it provides the means of extracting energy and nutrients from the diet while refueling at stopover sites, and it also serves as a source of lean mass that is catabolized in flight. After arrival at a stopover site, the digestive system may need to be reconstituted before maximal refueling rates can be attained (Hume and Biebach 1996; Karasov and Pinshow 2000). In association with these alternating demands, the digestive organs of long-distance migrants exhibit pronounced phenotypic flexibility, the rapid, reversible, and repeatable modulation of organ size or function (Piersma and Lindström 1997), during migration (Piersma 1998; McWilliams and Karasov 2001).

The digestive system is a good model for evaluating refueling capacity because it is responsive to changes in the quantity, composition, and quality of the diet (Karasov 1996; McWilliams and Karasov 2001). The digestive system is also an energetically expensive organ system to maintain (Starck 1996, 1999), and it is expected to exhibit economical design (Diamond and Hammond 1992). Maximal refueling rates are achieved primarily by hyperphagia (Karasov 1990); however, diet composition and quality are also important (Bairlein 1998). The primary response of the digestive system to periods of hyperphagia is an increase in size, which results in an increase in volumetric digestive capacity (Karasov 1996). When diet composition changes or diet quality increases, the rate of nutrient assimilation can be increased by modulating the activities of inducible digestive enzymes (Karasov 1996; McWilliams and Karasov 2001). Dietary modulation studies in birds provide general support for the hypothesis that the major pancreatic and intestinal digestive enzymes are modulated in relation to their dietary substrate levels (reviewed in Karasov 1996; Sabat et al. 1998; Levey et al. 1999; Caviedes-Vidal et al. 2000). Inducible digestive enzymes may, therefore, provide information on diet composition or quality (Karasov and Hume 1997).

Digestive capacity is an important determinant of refueling rate, and two important indices of digestive capacity are digestive system size and total hydrolytic capacity. We define the

digestive system as consisting of the proventriculus, gizzard, pancreas, and small intestine because the primary function of these organs is associated with the breakdown and assimilation of dietary nutrients. Although the esophagus, ceca, and large intestine contribute to the overall size of the alimentary canal, they are not included in our definition of the digestive system. The esophagus may provide limited space as a storage organ for ingested food; however, it has limited secretory activity (Duke 1986). The primary roles of the ceca and large intestine are related to microbial digestion and water absorption, respectively (Duke 1986). We use the size of the digestive system (excluding the pancreas) as an index of volumetric capacity. We assess enzymic capacity by measuring the activities of a series of enzymes that are involved in the breakdown of the diet's structural (e.g., chitin) and nutritional (fat, carbohydrate, and protein) components.

Proventricular chitinase (EC 3.2.1.14; Bairoch 1993) is an important component of the digestive machinery of birds that consume arthropods because their exoskeletons are composed largely of chitin (Place 1996*a*). The primary benefit of chitinolysis appears to be an increase in the accessibility of the nutrients contained in or concealed by the exoskeleton (Jackson et al. 1992). The pancreatic lipases (pancreatic lipase, EC 3.1.1.3, and nonspecific carboxyl ester lipase, EC 3.1.1.1) hydrolyze triglycerides into fatty acids and glycerol in the lumen of the small intestine, but full lipolytic capacity is bile dependent in birds (Place 1992). Bile salts emulsify dietary triglycerides, allowing the pancreatic lipases to efficiently hydrolyze fatty acids at the lipid-water interface (Place 1996*b*). Pancreatic amylase (EC 3.2.1.1) hydrolyzes α-1-4-glucosidic bonds of complex soluble carbohydrates, and one of the primary products is maltose (Alpers 1987), which is hydrolyzed to glucose by maltase (EC 3.2.1.20), an intestinal disaccharidase. Aminopeptidase-N (EC 3.4.11.2; also known as leucine-aminopeptidase and amino-oligopeptidase [Vonk and Western 1984]), an intestinal dipeptidase, has broad specificity in hydrolyzing oligopeptides into amino acids and accounts for almost all of the peptidase activity of the brush border membrane (Maroux et al. 1973).

We examined seasonal and age-related variation in digestive organ sizes and enzyme activities in refueling western sandpipers (*Calidris mauri* Cabanis), a small-bodied (22–35 g) long-distance migrant that breeds mainly in subarctic Alaska and overwinters primarily on the Pacific coast, between California and Peru (Wilson 1994). Western sandpipers use a short-hop migration strategy to move between coastal stopover sites en route to and from the breeding grounds (Wilson 1994; Iverson et al. 1996), and the spring migration is somewhat more protracted than fall migration. In fall, the juveniles initiate their first southward migration after completing growth; this delays the onset of migration by approximately 1 mo relative to the adults (Wilson 1994). Western sandpipers are considered to be invertebrate generalist and are known to consume a variety of benthic invertebrates, primarily arthropod crustaceans, polycheate annelids, and bivalve molluscs, while refueling at coastal stopover sites (Wilson 1994). The digestive system of adult western sandpipers attains its maximal size in refueling migrants; however, the digestive system of the later-migrating juveniles is substantially larger than the adults' (Guglielmo and Williams 2003). Age-dependent differences in digestive physiology may influence refueling rate and, thereby, the tempo or duration of migration; however, the functional significance of this age-dependent difference in digestive system size is unclear. For example, it is not known whether the juvenile's larger digestive system is simply an enlarged version of the adult's or if it is composed differently. Similarly, it is not known whether the enzymic capacity of the juvenile's larger digestive system is higher, lower, or equal to the adult's. We evaluate these alternatives through age-related comparisons of (1) the size of the digestive system and its component organs; (2) the standardized and total activities of five digestive enzymes; and (3) an index of the residual dietary energy contained in feces.

To place these age-related comparisons in the context of migration during the annual cycle, we also examine seasonal variation in the size of the digestive system and the activities of digestive enzymes in adults. To enhance our interpretation of the seasonal comparisons, we predict the relationships between three pairs of inducible digestive enzymes including gallbladder bile and then examine the validity of these predictions for refueling adult western sandpipers. First, we predict a positive correlation between pancreatic lipase and gallbladder bile because bile salts are essential to the efficient utilization of dietary fats (Place 1992, 1996*b*). Second, we predict a positive correlation between pancreatic amylase and intestinal maltase because the substrate of maltase, maltose, is one of the primary products of the hydrolysis of complex soluble carbohydrates by amylase (Alpers 1987). Third, we predict a positive correlation between two intestinal enzymes, maltase and aminopeptidase-N. Although maltase and aminopeptidase-N utilize rather different substrates, Sabat et al. (1998) reported a positive correlation between these enzymes in two species of passerine birds.

Material and Methods

Fieldwork and Sample Collection

Sandpipers were captured with mist nets (1 1/4 inch mesh, Avinet, Dryden, NY) and collected in accordance with permits from Environment Canada. Animal handling protocols were approved by the Simon Fraser University Animal Care Committee (B529) and conformed to the Canadian Committee for Animal Care Guidelines. Refueling sandpipers were captured at Boundary Bay, British Columbia, Canada (49°10′N, 123°05′W) during the fall migration of 1999 and the spring and fall migrations of 2000. Immediately after capture each bird was weighed (capture mass; ± 0.001 g) and culmen length was measured to assign sex: males $\leq$ 24.2 mm $<$ unknown sex $<$ 24.8 mm $\leq$ females (Page and Fearis 1971). During

the fall migrations of 1999 and 2000, fecal samples were collected from refueling sandpipers to determine the amount of residual dietary organic matter, which would have contained any residual dietary energy that was excreted. In 1999, fecal samples were collected from 19 sandpipers: 4 males (1 adult and 3 juveniles), 7 females (5 adults and 2 juveniles), and 8 unknown-sex birds (1 adult and 7 juveniles). In 2000, fecal samples were collected from 26 sandpipers: 14 males (8 adults and 6 juveniles), 9 females (2 adults and 7 juveniles), and 3 unknown-sex birds (2 adults and 1 juvenile). Fecal samples were collected by placing individual sandpipers in ventilated plastic containers fitted with raised hardware cloth bottoms for 30–40 min. Fecal samples were rinsed from the containers with distilled water and frozen for subsequent analysis. During the spring and fall migrations of 2000, 53 refueling female sandpipers were collected to obtain organ samples for enzyme assays: 13 spring adults (May 1–9), 22 fall adults (July 4–28), and 18 fall juveniles (August 9–27). Collected individuals were transported to Simon Fraser University for processing within 2 h of capture.

Immediately before dissection, culmen and tarsus were measured with digital calipers (±0.01 mm), and each bird was reweighed (dissection mass; ±0.001 g). Birds were killed by an overdose (4 mL/25 g) of a 1 : 1 mixture of ketamine hydrochloride (100 mg/mL) and xylazine (20 mg/mL) administered via intramuscular injection into the breast muscle. Birds were dissected immediately after death to collect gallbladder bile and the digestive organs. Bile was extracted from the gallbladder with a sterile 1-mL tuberculin syringe. The proventriculus was separated from the esophagus distal to the bifurcation of the trachea and at its connection with the gizzard. The small intestine was separated from the gizzard at the pylorus and from the large intestine immediately proximal to the ceca. The pancreas was delicately separated from the duodenal loop of the small intestine. Gastroliths and grit were removed from the gizzard by cutting it open and rinsing it with ice-cold physiological saline (350 mOsm/kg H_2O). Digesta was purged from the lumen of the small intestine by suturing a gavauge needle to its proximal end and gently flushing the contents with ice-cold physiological saline. The evacuated small intestine was cut in half at Meckel's diverticulum, and each resulting section was cut in half again; this resulted in four intestinal sections: the duodenum and the proximal, mid, and distal ileum. After excision, the organs were rinsed in ice-cold physiological saline, adherent fat and mesenteries were removed, and the organs were blotted dry, weighed (±0.001 g), and flash frozen (liquid N_2, −196°C) in labeled cryovials.

At the end of each dissection the sex of the bird was verified, keel length was measured with digital calipers (±0.01 mm), and the carcass was stored at −20°C. For proventricular chitinase, sample sizes were reduced by 2 for spring adults and by 1 for fall adults due to lost tissue. Total gallbladder bile was not reported because bile was lost from several individuals during collection. For intestinal maltase and aminopeptidase-N, sample size was reduced by 4 for the fall juveniles because the intestines from these birds were used for histological studies by Stein and Williams (2003).

Measurement of Proventricular Chitinase

Protein Extraction and Assay. Individual proventriculi were homogenized for 30 s using an OMNI 5000 homogenizer, setting 6, in 10 mL of a 1.0 M acetate buffer, pH 4.5. Homogenates were centrifuged at 10,000 *g* for 10 min, and the supernatant was stored at −80°C until it was assayed. Proventricular protein, used to standardize chitinase activity, was determined using a Pierce BCA (bicinchoninic acid) kit, adapted for use in microtiter plates. Absorbance was recorded at 590 nm on a microplate reader (Vmax; Molecular Devices, Menlo Park, CA) using bovine serum albumin as a standard. Assays were performed in triplicate, with a mean coefficient of variation of 3.5%.

Chitinase Activity Assay. Chitinase activity was measured using the tritiated chitin method of Molano et al. (1977) with modifications by Cabib and Sburlati (1988). Proventricular extracts were thawed on ice, and 100 μL was diluted 1 : 10 with the 1.0 M acetate buffer, pH 4.5. The reaction mixture consisted of 70 μL distilled water, 15 μL of suspended acetyl-[^{3}H]-chitin (0.5 μCi/mg, 0.23 μCi/μM N-acetyl-D-glucosamine [NAG]), 5.0 μL of the 1.0 M acetate buffer, and 10 μL of diluted proventricular extract. The reaction was initiated by the addition of the proventricular extract, incubated at 41°C on a shaker for 60 min, and stopped by the addition of 300 μL of 10% (w/v) trichloroacetic acid. The suspension was centrifuged for 5 min at 500 *g*, and 200 μL of the supernatant was transferred to a scintillation vial. Radioactivity (dpm) was determined with a Beckman LS6500 autoanalyzer using the window preset for tritium. Activity was calculated from dpm, which was due to the liberation of tritiated NAG. Assays were performed in triplicate, with a mean coefficient of variation of 6.8%. Chitinase activity is reported as total activity (μg NAG/min) and as specific activity (μg NAG/min/mg protein).

Analysis of Gallbladder Bile

Bile samples were thawed on ice and centrifuged at 20,000 *g* at 4°C for 10 min. Biliary protein was precipitated by combining 10 μL of bile with 10 μL of methanol, and the resulting methanolic bile was diluted 1 : 50 with distilled H_2O. Bile salt concentrations were assayed using 3 α-hydroxysteroid dehydrogenase (E.C. 1.1.150; Coleman et al. 1979). The reaction mixture contained 170 μL of a hydrazine buffer (33 mM sodium pyrophosphate and 0.33 M hydrazine sulfate), pH 9.5, 20 μL of 0.33 mM NAD^+, and 5 μL of diluted methanolic bile. The reaction was started by the addition of 5 μL (0.2 Units)

of 3 α-hydroxysteroid dehydrogenase in a potassium phosphate buffer, pH 7.5. After a 1-h incubation at 25°C, absorbance was recorded at 340 nm on a microplate reader at 25°C with taurocholate as the standard. Concentration was calculated from the increase in absorbance at 340 nm, which was due to the production of NADH. Bile salt assays were performed in duplicate with a mean coefficient of variation of 3.4%.

Measurement of Pancreatic Hydrolytic Enzymes

Protein Extraction and Assay. Each pancreas was homogenized for subsampling by powdering it in a stainless steel mortar and pestle that was partially submerged in liquid N_2 (−196°C). The resulting powder was placed in a labeled cryovial and frozen at −80°C until extraction. For amylase and lipase activity measurements, subsamples of the powdered pancreas, 10–0 mg, were extracted in 1–3 mL of a 10 mM sodium acetate buffer, pH 4.8 (buffer volume depending on tissue mass), for 15 min at 4°C. The buffer contained 0.9% (w/v) NaCl, 0.2% (w/v) Triton X-100, 3 mM sodium taurocholate, 2 mM hydrocinnamic acid, and 1 mM benzamidine. The extract was sonicated (Branson model 460) on ice for 1 min at 50% power and 50% duty cycle with a microtip and then centrifuged at 20,000 *g* for 20 min at 4°C. The supernatant was removed and frozen at −80°C until it was assayed. Total pancreatic protein in each extract, used to standardize amylase and lipase activity, was determined using a Pierce BCA kit, adapted for use in microtiter plates. Absorbance was recorded at 590 nm on a microplate reader using bovine serum albumin as a standard. Protein assays were performed in triplicate, with a mean coefficient of variation of 6.4%.

Lipase Activity Assay. Lipase activity was measured at 41°C and pH 8.0 using a pH-stat (TTT80 Radiometer) with olive oil, a long-chain triglyceride, as the substrate and 0.02 N NaOH as the neutralizing base. Long-chain fatty acids (pk ~ 6.8) are incompletely ionized below pH 9.0; consequently, lipolytic activity is underestimated at pH 8.0 when a long-chain triglyceride is the substrate. Although pH 8.0 is more realistic physiologically than pH 9.0, the reported activities are not absolute. Titrametric assay of bile-dependent lipolytic activity was performed using a 0.5% (w/v) gum arabic–stabilized emulsion of olive oil, which was sonicated for 3 min at a power setting of 30 on a Branson sonicator immediately before use. The 10-mL assay volume contained 0.5 mL of substrate emulsion in a 2 mM tris-maleate buffer (pH 8.0), 150 mM NaCl, 1 mM $CaCl_2$, and 0.02% mM sodium azide. Nonenzymatic base uptake was measured by incubating the reaction mixture for 2 min without enzyme. The reaction was initiated by the addition of 50 μL of 200 mM sodium taurocholate, a common avian bile salt, followed by 100 μL of proventricular extract. The presence of bile salts fully activates bile-dependent carboxyl-ester lipase and the colipase-pancreatic lipase. Preliminary studies demonstrated that maximal activation by taurocholate occurred at concentrations above 1.0 mM. Activity was calculated from the amount of base required to neutralize liberated fatty acids and maintain pH 8.0. Assays were performed in duplicate with a mean coefficient of variation of 1.6%. Lipase activity is reported as total activity (μM/min) and as specific activity (μM/min/mg protein).

Amylase Activity Assay. Amylase activity was determined with an amylase assay kit (Sigma Diagnostics). The reaction was conducted at pH 7.0, using 4,6-ethylidine (G_7)-*p*-nitrophenyl (G_1)-α, D-maltoheptaside as a substrate, which results in α-glucosidic release of *p*-nitrophenol. The reaction was initiated by adding 5 μL of pancreatic extract, diluted 1 : 10, to 200 μL of reagent. After 2 min, the rate of increase in absorbance at 405 nm is directly proportional to amylase activity. After allowing the reaction to equilibrate for 2 min, absorbance was recorded continuously at 405 nm for 5 min at 41°C on a microplate reader. Activity was calculated from the mean rate of increase in absorbance at 405 nm, which was due to the production of *p*-nitrophenol. Amylase activity is reported as total activity (μM/min) and as specific activity (μM/min/mg protein).

Measurement of Intestinal Hydrolytic Enzymes

Tissue Homogenates and Standardization. The entire small intestine was homogenized for 30 s using an OMNI 5000 homogenizer, setting 6, in 6–14 mL of 350-mM mannitol in 1 mM Hepes/KOH, pH 6.5 (buffer volume depending on tissue mass). The final volume of the homogenate was calculated as the sum of intestine mass (g) and buffer volume (mL), assuming a density of 1.0 (g/mL) for the small intestine. For three fall adults and three fall juveniles, the four intestinal sections were homogenized separately and were used to examine variation in enzyme activity along the length of the small intestine. Intestinal enzyme assays were conducted using tissue homogenates rather than isolated vesicles because yields and activities of membrane preparations are often low and variable (Martínez del Rio et al. 1995 and references therein). Intestinal enzyme activities were calculated on the basis of absorbance standards constructed for glucose and *p*-nitroanilide. Martínez del Rio et al. (1995) provide justification for our choice of standardization.

Maltase Activity Assay. Maltase activity was measured according to the methodology of Dahlqvist (1964), with modifications by Martínez del Rio et al. (1995). Assay mixtures, 56 mM, were prepared by adding 0.202 g maltose to 10 mL of maleate/NaOH, pH 6.5. The stop-development reagent was a mixture of 250 mL of 0.5 M monobasic/dibasic phosphate buffer (pH 6.5), 250 mL 1 M Tris/HCl (pH 6.5), and one 500-mL bottle of glucose (Trinder) reagent powder (Sigma Diagnostics). Tissue

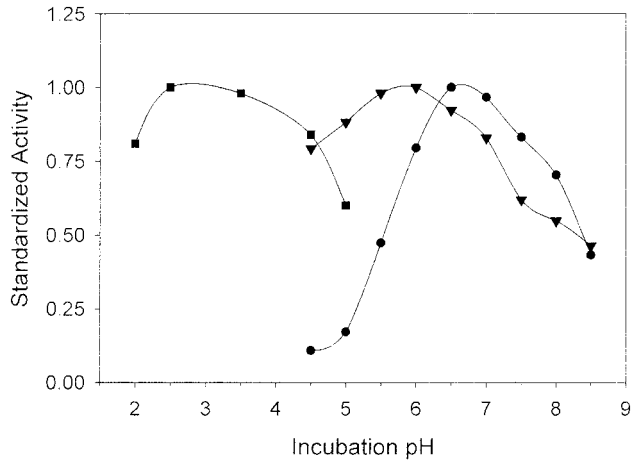

Figure 1. From the western sandpiper, pH_{optima} of proventricular chitinase (*squares*), intestinal maltase (*triangles*), and intestinal aminopeptidase-N (*circles*).

homogenates were thawed and then diluted 1 : 50 in 350 mM mannitol in 1 mM Hepes/KOH, pH 6.5. To start the reaction, 100 μL of diluted homogenate was combined with 100 μL of 56 mM maltose, and then the tube was vortexed and incubated at 40°C. After 15 min, 3.0 mL of the stop-development reagent was added, and the reaction mixture was allowed to develop for 20 min at room temperature. Absorbance was measured at 505 nm in a Beckman DU-64 spectrophotometer. Activity was calculated from the absorbance at 505 nm, which was due to the production of red chinonimin dye from the Trinder reaction. Assays were performed in duplicate, with a mean coefficient of variation of 2.6%. Maltase activity is reported as total activity (μM/min) and as tissue-specific activity (μM/min/g tissue).

Aminopeptidase-N Activity Assay. Aminopeptidase-N activity was determined using the methodology of Roncari and Zuber (1969) with L-alanine-*p*-nitroanilide as the substrate. A 2.04 mM L-alanine-*p*-nitroanilide assay mixture was prepared by dissolving 125 mg L-alanine-*p*-nitroanilide in double distilled water, 250 mL final volume. The dissolved substrate was combined with 250 mL of 0.2 M phosphate buffer, pH 6.5. Tissue homogenates were thawed on ice, and 10 μL of undiluted homogenate was combined with 1.0 mL of the L-alanine-*p*-nitroanilide assay mixture at 40°C. The tube was vortexed and then incubated at 40°C for 15 min. The reaction was stopped by the addition of 3.0 mL of ice-cold 2N acetic acid and vortexing. Absorbance was measured at 384 nm in a Beckman DU-64 spectrophotometer. Activity was calculated from the increase in absorbance at 384 nm, which was due to the production of *p*-nitroanilide. Assays were performed in triplicate, with a mean coefficient of variation of 2.5%. Aminopeptidase-N activity is reported as total activity (μM/min) and as tissue-specific activity (μM/min/g tissue).

Determination of pH_{optima}

The optimal reaction pH for proventricular chitinase and those for intestinal maltase and aminopeptidase-N were determined by running the assays as described above, while varying the pH. The resulting activities were standardized to the enzyme's maximal activity to facilitate presentation (Fig. 1). The pH_{optima} for each enzyme was determined by fitting a quadratic to the data, taking the derivative of the fitted equation, and solving for the positive pH value where the slope was equal to 0. The chitinase $pH_{optima} = 3.1$, the maltase $pH_{optima} = 5.8$, and the aminopeptidase-N $pH_{optima} = 6.9$. The activities reported for these three enzymes are adjusted to their pH_{optima} and, therefore, represent maximal values.

Fecal Analysis

In birds, the luminal contents of the small intestine and ceca mix with urinary waste in the large intestine, and they are excreted together. To isolate organic material of dietary origin from feces, endogenously produced uric acid must first be removed. Uric acid was removed from the fecal samples by adding 20 mL of a 0.1 M glycine buffer, pH 9.3, which dissolves uric acid (Adeola and Rogeler 1994); the samples subsequently were vortexed and centrifuged at 5,000 rpm for 10 min, and the supernatant was removed. Each sample was rinsed with 20 mL distilled water and centrifuged at 5,000 rpm for 10 min, and the supernatant was removed again. The sample collection and subsequent removal of uric acid would result in the removal of soluble organic material; therefore, it is likely that only the insoluble organic material of dietary origin was retained. The sample was dried to constant mass at 105°C in an air-circulation oven and weighed. Ash-free dry mass was determined by oxidizing organic material to carbon dioxide and ash by igniting the samples in a muffle furnace at 450°C for 4 h and reweighing the ignited sample (Speakman 1987). Due to the loss of soluble organic material, ash-free dry mass should be interpreted as an index of residual dietary organic matter.

Statistical Analyses

A multivariate measure of structural size was generated from a principal components analysis (PCA) of culmen, tarsus, and keel lengths (Rising and Somers 1989). The first principal component (PC1) explained 53% of the total variation in these univariate measures of structural body size, which had high positive loadings (culmen = 0.58, tarsus = 0.59, and keel = 0.56) on PC1 (eigenvalue = 1.58). Body mass fluctuates dramatically during migration; to control for variation in body condition among migrants, we used the residuals from a re-

Table 1: Wet mass of female western sandpipers refueling during migration at Boundary Bay, British Columbia

Variable	Spring Adults	Fall Adults	Fall Juveniles
Body mass (g)	26.0 ± .6*	29.4 ± .7	27.5 ± .7
Digestive system (g)	2.38 ± .07	2.35 ± .05	2.58 ± .05*
Proventriculus (mg)	101 ± 5	115 ± 4	84 ± 4*
Gizzard (mg)	770 ± 27	726 ± 21	790 ± 22
Pancreas (mg)	211 ± 9	222 ± 7	214 ± 7
Small intestine (g)	1.30 ± .05	1.29 ± .04	1.49 ± .04*
n	13	22	18

Note. Body mass is capture mass, and values are means ± SE. Digestive system and organ masses are least squares means ± SE, with body mass minus digestive system or organ mass as the covariate.

* Significant difference from fall adults; $P < 0.025$.

gression of dissection mass on structural size, PC1 ($F_{1,51} = 4.74$, $P < 0.05$).

Significant seasonal or age-related differences in organ sizes and enzyme activities were identified with a post hoc procedure recommended by Stevens (1996). Briefly, a significant overall multivariate analysis of variance (MANOVA) or covariance (MANCOVA) including the three groups of migrants was followed by two preplanned pair-wise multivariate comparisons, one between spring and fall adults and the other between fall adults and juveniles, with $\alpha = 0.05$ in each case. Significant pair-wise multivariate tests were followed by a series of univariate ANOVAs or ANCOVAs. In these univariate analyses the two preplanned comparisons were made using contrast statements, and experiment-wise error was controlled at $\alpha = 0.05$ by Bonferroni correction (adjusted $\alpha = 0.025$). To avoid part-whole correlation in the ANCOVAs for organ sizes, body mass at dissection minus organ mass was used as a covariate (Christians 1999). Predicted relationships between standardized enzyme activities were examined with correlation analysis and, due to age-related differences in gut structure, only the data from adults were used. Repeated-measures ANOVA was used to examine variation in enzyme activities along the length of the small intestine. Fecal sample dry mass was analyzed with ANOVA, and ash-free dry mass was analyzed with repeated-measures ANCOVA, with fecal sample dry mass as a covariate. None of the variables had sample distributions that deviated systematically from normal (Shapiro-Wilks test, $P > 0.05$). Statistical analyses were conducted in SAS (SAS Institute 1990).

Results

Body Size and Body Mass

Structural size (PC1) was independent of season and age ($P \geq 0.17$). Among the adults, spring migrants were 12% lighter at capture than fall migrants (Table 1; $F_{1,50} = 11.24$, $P < 0.01$); however, capture mass was independent of age among fall migrants ($P \geq 0.05$). Capture mass and dissection mass were positively correlated ($r = 0.99$, $P < 0.0001$, $n = 53$), and dissection mass was used to generate the body condition covariate.

Organ Sizes

Relative organ sizes varied significantly among the three groups of migrants (Wilks's $\lambda = 0.32$, $P < 0.0001$, $n = 53$; body condition was the covariate). Relative organ sizes were independent of season among adults (Wilks's $\lambda = 0.81$, $P = 0.17$, $n = 40$); however, relative organ sizes varied with age among fall migrants (Wilks's $\lambda = 0.30$, $P < 0.0001$, $n = 35$). Among the fall migrants, the digestive system (wet mass) was 10% larger in juveniles (Table 1; $F_{1,49} = 9.62$, $P < 0.01$), primarily because their small intestine was 16% larger than the adults' ($F_{1,49} = 12.50$, $P < 0.001$). In contrast, the juveniles' proventriculi were 27% smaller than the adults' ($F_{1,49} = 30.30$, $P < 0.0001$). Gizzard and pancreas mass were independent of age among fall migrants ($P \geq 0.05$).

Enzyme Activities

Relationships between Enzymes. Pancreatic lipase-specific activity and gallbladder bile concentration were positively correlated (Fig. 2*A*; $r = 0.38$, $P = 0.025$, $n = 34$). Pancreatic amylase-specific activity and intestinal maltase tissue-specific activity were uncorrelated (Fig. 2*B*; $r = -0.31$, $P = 0.069$, $n = 35$). Intestinal maltase and aminopeptidase-N tissue-specific activities were positively correlated (Fig. 2*C*; $r = 0.58$, $P < 0.001$, $n = 35$). In addition, tissue-specific activities of maltase and aminopeptidase-N decreased 79% (Fig. 3; $F_{3,15} = 35.47$, $P < 0.0001$) and 29% ($F_{3,15} = 8.39$, $P < 0.001$), respectively, between the duodenum and distal ileum.

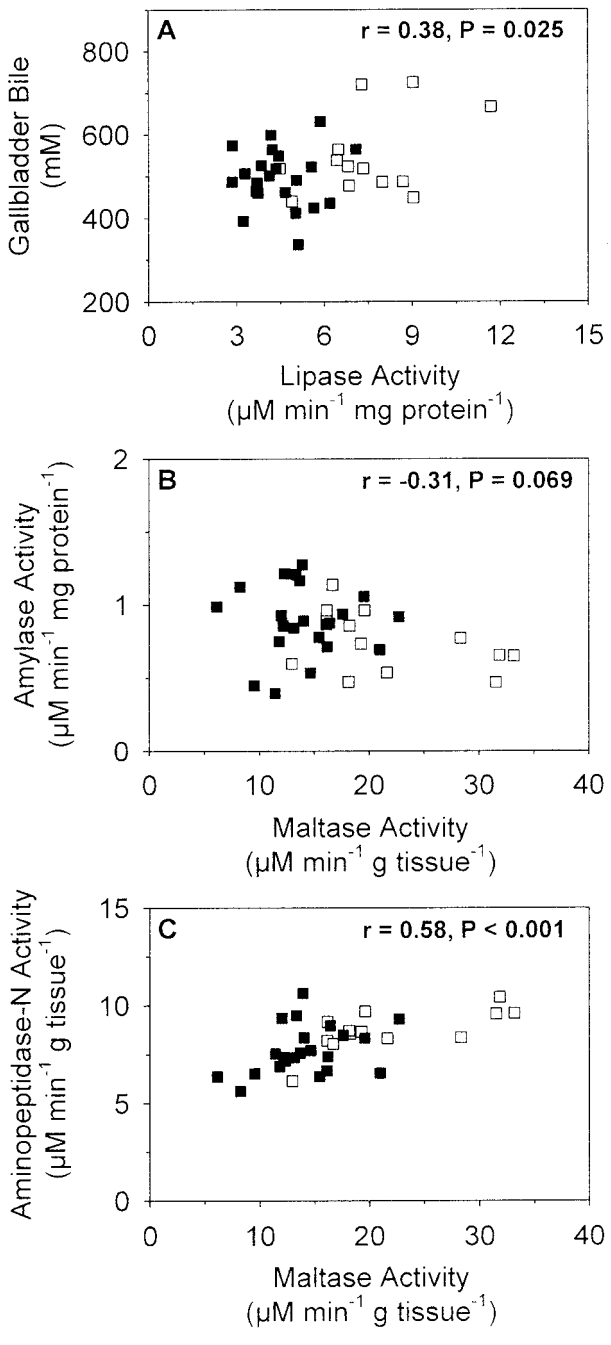

Figure 2. Relationships between pancreatic lipase and gallbladder bile (*A*), pancreatic amylase and intestinal maltase (*B*), and intestinal maltase and aminopeptidase-N (*C*) for adult female western sandpipers refueling during the spring (*open squares*) and fall (*filled squares*) migrations at Boundary Bay, British Columbia.

Standardized Enzyme Activities. Standardized enzyme activities varied significantly among the three groups of migrants (Wilks's $\lambda = 0.18$, $P < 0.0001$, $n = 45$). Standardized enzyme activities varied seasonally among adults (Wilks's $\lambda = 0.31$, $P < 0.0001$, $n = 31$) and with age among fall migrants (Wilks's $\lambda = 0.55$, $P < 0.01$, $n = 34$). Among the adults, spring migrants had 67% higher lipase-specific activity (Fig. 4*D*; $F_{1,50} = 45.74$, $P < 0.0001$), 56% higher tissue-specific maltase activity (Fig. 4*E*; $F_{1,46} = 18.97$, $P < 0.0001$), and 13% higher tissue-specific aminopeptidase-N activity than fall migrants (Fig. 4*F*; $F_{1,46} = 6.20$, $P < 0.025$). Chitinase-specific activity, bile concentration, and amylase-specific activity were independent of season among adults (Fig. 4*A*–4*C*; $P \geq 0.06$). Among the fall migrants, juveniles had 19% lower tissue-specific aminopeptidase-N activity than adults (Fig. 4*F*; $F_{1,46} = 13.88$, $P < 0.001$). Chitinase-specific activity, bile concentration, lipase- and amylase-specific activities, and maltase tissue-specific activity were independent of age among fall migrants (Fig. 4*A*–4*E*; $P \geq 0.05$).

Total Enzyme Activities. There was significant variation in total enzyme activities among the three groups of migrants (Wilks's $\lambda = 0.30$, $P < 0.0001$, $n = 45$). Total enzyme activities varied seasonally among adults (Wilks's $\lambda = 0.38$, $P < 0.0001$, $n = 31$) and with age among fall migrants (Wilks's $\lambda = 0.70$, $P = 0.05$, $n = 34$). Among the adults, spring migrants had 22% higher total lipase (Table 2; $F_{1,50} = 5.34$, $P < 0.025$) and 47% higher total maltase ($F_{1,46} = 14.89$, $P < 0.001$) activities than fall migrants; however, spring migrants also had 37% lower total amylase ($F_{1,50} = 6.49$, $P < 0.01$) activity. Total chitinase and aminopeptidase-N activities and were independent of season among adults ($P \geq 0.18$). Among the fall migrants, juveniles had 20% lower total chitinase activity ($F_{1,47} = 7.20$, $P < 0.01$) and 23% lower total lipase activity ($F_{1,50} = 7.30$, $P < 0.01$) than adults. Total amylase, maltase, and aminopeptidase-N activities were independent of age among fall migrants ($P \geq 0.22$).

Fecal Analysis

Assessment of a sex-related effect on fecal sample dry mass and ash-free dry mass was restricted to data from known sex birds (males $n = 18$ and females $n = 16$). Fecal sample dry mass ($F_{1,32} = 1.47$, $P = 0.235$) and ash-free dry mass ($F_{1,31} = 1.39$, $P = 0.247$) were independent of sex. The data from males and females were pooled for subsequent analysis, and data from 11 unknown sex birds were included also. Fecal dry mass ($F_{1,43} = 0.90$, $P = 0.349$) and ash-free dry mass ($F_{1,42} = 0.45$, $P = 0.504$) also were independent of year. For subsequent analyses, the data from 1999 and 2000 were pooled. Fecal sample dry mass was practically identical for adults and juveniles (Table 3; $F_{1,43} = 0.00$, $P = 0.981$); however, fecal samples from juveniles had a higher proportion of ash-free dry matter than the adult's ($F_{1,42} = 5.47$, $P < 0.025$), approximately 10%.

Discussion

One of our primary goals was to better understand the functional significance of an enlarged digestive system to juvenile

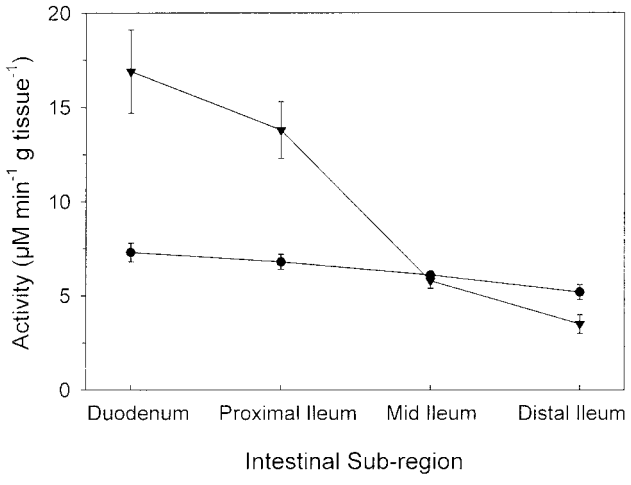

Figure 3. Variation in maltase (*triangles*) and aminopeptidase-N (*circles*) activities along the length of the small intestine of female western sandpipers refueling during fall migration at Boundary Bay, British Columbia.

western sandpipers making their first southward migration. To place these age-related comparisons in the context of migration during the annual cycle, we also examined seasonal variation in digestive system and organ size and digestive enzyme activities in adult migrants. The digestive system of adult western sandpipers increases in size after the initiation of migration and attains its maximal size in refueling migrants, independent of season (Stein 2002; Guglielmo and Williams 2003). Consistent with this, the relative size of the digestive system and its component organs was similar in adult migrants during spring and fall. Although there was no seasonal variation in the size of the digestive system or organs, adult sandpipers exhibited marked seasonal variation in pancreatic and intestinal enzyme activities. Spring migrants had higher standardized and total pancreatic lipase activities but lower total amylase activity than fall migrants. Spring migrants also had higher total intestinal maltase activity and higher standardized activities for maltase and aminopeptidase-N. The seasonal differences in enzyme activities suggest that diet quality was lower during the fall stopover period. During fall migration, the digestive system of the later-migrating juveniles was substantially larger than the adults'. The juvenile's digestive system was not simply an enlarged version of the adult's; it was characterized by a smaller proventriculus and a larger small intestine. The juvenile's larger digestive system was associated with lower total enzymic capacity, due primarily to lower total chitinase and lipase activities. Fecal samples from juveniles contained a higher proportion of organic material than those from adults. These results demonstrate additional age-related differences in digestive system structure and function that could influence the tempo and duration of migration for juveniles.

Relationships between Enzymes

We predicted positive correlations between the standardized activities of three pairs of inducible digestive enzymes including gallbladder bile. The predicted correlations between pancreatic lipase and gallbladder bile and between pancreatic amylase and intestinal maltase are based on functional relationships; full lipolytic activity is bile dependent in birds (Place 1992), and amylase and maltase catalyze sequential reactions in the hydrolysis of complex soluble carbohydrates (Alpers 1987). Pancreatic lipase and gallbladder bile were positively correlated, as predicted. This suggests that lipase and bile may be coregulated in refueling western sandpipers for the efficient utilization of dietary fats. Contrary to prediction, however, pancreatic amylase and intestinal maltase were not correlated. This result was surprising because some of the benthic invertebrates that western sandpipers prefer, particularly small bivalves (Senner et al. 1989), store energy as glycogen rather than as triglycerides (Beukema 1997). The most likely explanation for the absence of the predicted correlation is that modulation of pancreatic enzymes occurs more quickly (24 h; Imondi and Bird 1967) than that of intestinal enzymes (48–96 h; Biviano et al. 1993). One important consequence of this temporal difference in enzyme induction is that pancreatic enzymes should reflect a more recent dietary history than intestinal enzymes, and this is particularly important when diet composition changes, which may occur when birds move between stopover sites.

Although maltase and aminopeptidase-N utilize rather different substrates, Sabat et al. (1998) reported a strong positive correlation between these intestinal enzymes in two species of passerine birds. Therefore, the predicted correlation between intestinal maltase and aminopeptidase-N is based on a structural relationship; both of these enzymes are expressed in the brush border membrane of intestinal enterocytes (Karasov and Hume 1997) and not on a functional relationship. As predicted, intestinal maltase and aminopeptidase-N activities were positively correlated in refueling western sandpipers. Although there was a strong positive correlation between the activities of intestinal maltase and aminopeptidase-N measured in whole tissue homogenates, these enzymes exhibited distinct patterns of expression along the length of the small intestine. These results suggest that the expression of maltase and aminopeptidase-N may be coregulated either by nonspecific modulation, where the substrate of one of the enzymes leads directly or indirectly to changes in the activities of both enzymes (Karasov and Diamond 1983), or by changes in the ultrastructure of the small intestine that influence both enzymes. In passerine birds, maltase activity decreases distally in the small intestine and is independent of diet; however, aminopeptidase-N activity may increase, decrease, or remain constant along the length of the small intestine, depending on the species (Witmer and Martínez del Rio 2001). The pattern of aminopeptidase-N expression, therefore, may be indicative of dietary preference or speciali-

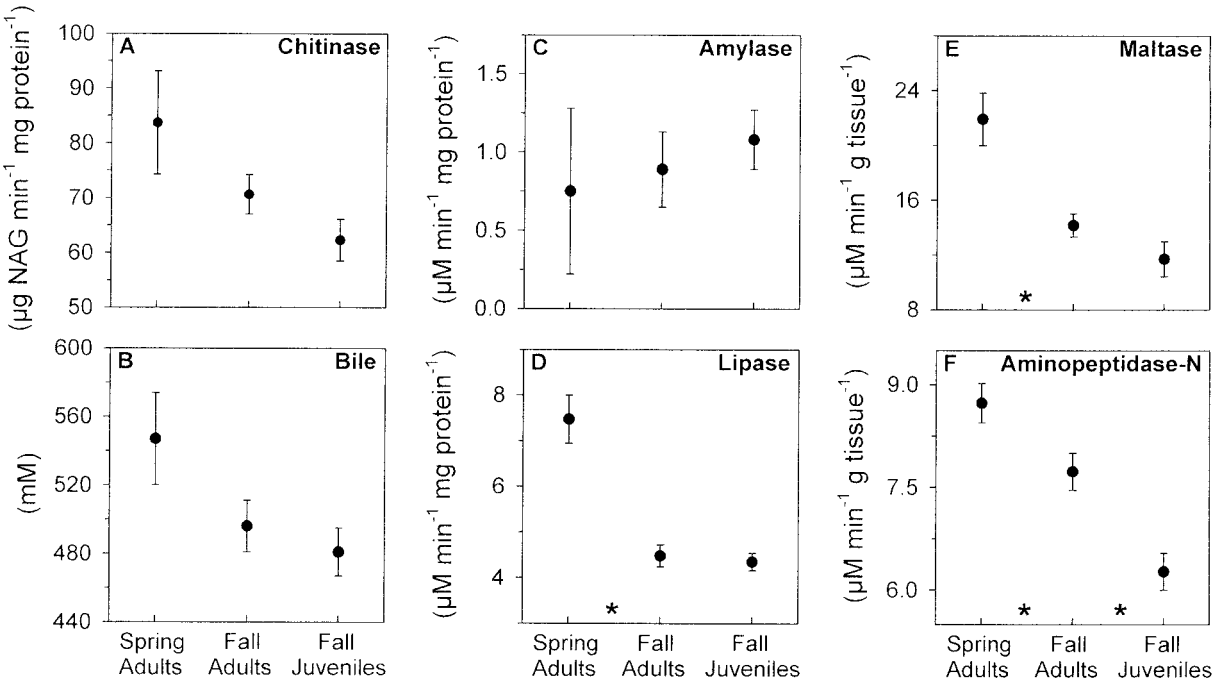

Figure 4. Standardized proventricular chitinase (*A*), gallbladder bile (*B*), pancreatic amylase (*C*), pancreatic lipase (*D*), intestinal maltase (*E*), and intestinal aminopeptidase-N (*F*) activities from female western sandpipers refueling at Boundary Bay, British Columbia. An asterisk indicates a significant difference from fall adults; $P < 0.025$.

zation (Witmer and Martínez del Rio 2001). In the western sandpiper, the pattern of aminopeptidase-N expression is similar to that of passerine birds that specialize on protein-rich invertebrates (Witmer and Martínez del Rio 2001), and this is consistent with what is known about the diet of the western sandpiper.

Seasonal Comparisons among Adults

Although western sandpipers are considered to be short-hop long-distance migrants (Wilson 1994), flight distance varies with individual and season (Butler et al. 1996; Iverson et al. 1996). Prebreeding adults arrive at Boundary Bay after a relatively short flight from as far south as San Francisco Bay, California; this 1,350-km flight would take approximately 16 h (Iverson et al. 1996). Postbreeding adults initiate southward migration with a nonstop flight across the Gulf of Alaska (Butler et al. 1996), returning to Boundary Bay after a 2,400-km flight, which would take 29 h (Lank et al. 2003). Despite the substantial difference in flight length and duration, the relative size of the digestive system and its component organs was remarkably similar in spring and fall adults on stopover at Boundary Bay. Since the digestive system of adult western sandpipers attains its maximal size during refueling (Stein 2002; Guglielmo and Williams 2003), it is likely that the intake rates of refueling adults are also at or near maximal (Karasov 1996).

Adult western sandpipers are thought to consume a similar array of benthic invertebrates while refueling at Boundary Bay during the spring and fall stopover periods; however, the available diet data are based on fecal analysis, and the sample sizes are limited (spring adults $n = 26$, fall adults $n = 20$; Seaman 2003). These data indicate that arthropod crustaceans (e.g., amphipods, ostracods, and copepods) are the most important prey during the spring and fall stopover periods (Seaman 2003). This is consistent with the observation that western sandpipers prefer to forage in areas with high densities of *Corophium* amphipods (Colwell and Landrum 1993) and confirms the importance of arthropod crustaceans as food for refueling sandpipers at Boundary Bay. Although the nutritional composition of the diet of western sandpipers refueling at Boundary Bay is not known, the nutritional composition and energy content of one of their preferred prey taxa, the *Corophium* amphipods, varies seasonally due to growth and reproduction (Boates and Smith 1979; Dobrzycka and Szaniawska 1993). The spring migration of western sandpipers appears to coincide with the reproductive season of the *Corophium* amphipods. In April and May, *Corophium* populations are composed primarily of gravid females, which have a higher energy density than nonreproducing females, due to a higher lipid content (Boates and Smith 1979; Dobrzycka and Szaniawska 1993). In July, when the postbreeding adult western sandpipers return to Boundary Bay, *Corophium* populations are composed primarily of nonreprod-

Table 2: Whole-organ enzyme activities of female western sandpipers refueling during migration at Boundary Bay, British Columbia

Enzyme	Spring Adults	Fall Adults	Fall Juveniles
Proventricular chitinase (μg NAG min^{-1} 10^3)	745 ± 45	849 ± 50	675 ± 46*
Pancreatic lipase (μM min^{-1})	148 ± 10*	121 ± 8	93 ± 5*
Pancreatic amylase (μM min^{-1})	15.8 ± 2.1*	24.9 ± 2.1	23.9 ± 2.8
Intestinal maltase (μM min^{-1})	27.2 ± 2.1*	18.4 ± 1.1	17.6 ± 1.8
Intestinal aminopeptidase-N (μM min^{-1})	10.9 ± .5	10.3 ± .5	9.4 ± .4
n	13	22	18

Note. Values are means ± SE.

* Significant difference from fall adults; $P < 0.025$. Sample sizes are as shown except for proventricular chitinase, $n = 11$ for spring adults and $n = 21$ for fall adults, and intestinal maltase and aminopeptidase-N, $n = 14$ for fall juveniles.

ucing adult females (Boates and Smith 1979). At Boundary Bay, western sandpipers preferentially select larger *Corophium* amphiods (Wolf 2001) and may be targeting gravid females.

Arthropod crustaceans such as the *Corophium* amphipods store energy as lipids, not carbohydrates, and invertebrates that use lipids exclusively as reserve materials show strong seasonal fluctuations in energy density (Beukema 1997). As a consequence, adult western sandpipers probably experience a seasonal change in diet composition or quality at Boundary Bay. Consistent with this idea, refueling adult western sandpipers exhibited marked seasonal variation in pancreatic and intestinal enzyme activities. Because of a more rapid time course of induction, pancreatic enzymes probably best reflect diet composition at Boundary Bay. The pancreatic enzymes may even reflect a beach-specific dietary signal because radiotagged western sandpipers refueling at Boundary Bay used the same beach for their entire stay (Butler et al. 2002). Spring migrants had higher standardized and total pancreatic lipase activities than fall migrants, which strongly suggests that the spring diet was enriched with lipids. Spring migrants also had lower total pancreatic amylase activity (Table 2), which suggests that the spring diet was low in glycogen. Together, these results suggest that the adult western sandpipers refueling at Boundary Bay in the spring were consuming a lipid-rich diet, probably more lipid-rich arthropod crustaceans than glycogen-rich bivalves. Since the energy content of lipids (38.9 kJ/g) is approximately twice that of carbohydrates (17.2 kJ/g) and protein (22.6 kJ/g, though the energy yield is 18.0 kJ/g for birds; Whittow 1986), the spring diet also may have had a higher energy content.

Table 3: Mass of fecal samples from western sandpipers refueling during fall migration at Boundary Bay, British Columbia

Fecal Sample	Adults	Juveniles
Dry mass (mg)	25.0 ± 3.3	25.1 ± 3.9
Ash-free dry mass (mg)	7.9 ± .5	9.5 ± .4*
Ash-free dry mass (%)	34.8 ± 3.2	44.6 ± 2.5
n	19	26

Note. Values are means ± SE.

* Significant difference; $P < 0.025$. Values are least square means ± SE, with fecal sample dry mass as a covariate. Percentage values are presented for clarity.

Intestinal enzymes probably reflect an earlier dietary history than pancreatic enzymes; in fact, the length of stay of western sandpipers at Boundary Bay may be too short to allow intestinal enzymes to adjust to local dietary conditions. Making inferences about diet composition based on maltase and aminopeptidase-N is further complicated by what appears to be a phylogenetic difference in regulatory mechanisms of these enzymes (reviewed in Caviedes-Vidal et al. 2000). In the passeriformes, aminopeptidase-N is modulated in response to its dietary substrate, but maltase is not; the anseriformes, and possibly the galliformes, show the opposite pattern (Caviedes-Vidal et al. 2000). Furthermore, two species of passerine birds exhibited a highly significant positive correlation between maltase and aminopeptidase-N (Sabat et al. 1998), and a similar relationship was evident in adult western sandpipers. The spring migrants had higher total and standardized maltase activities and higher standardized aminopeptidase-N activities than fall migrants. Low total pancreatic amylase activity in spring migrants suggests that their diet at Boundary Bay was probably low in complex soluble carbohydrates. It is possible that, like the passerine birds studied, dietary protein regulates the expression of both intestinal enzymes, but this is uncertain. Seasonal differences in intestinal maltase and aminopeptidase-N may be indicative of seasonal differences in diet composition or quality on a broader timescale than the pancreatic enzymes; however, they could also reflect seasonal changes in the ultrastructure of the small intestine that are associated with migration.

If the seasonal differences in pancreatic enzymes reflect a seasonal difference in diet quality at Boundary Bay, then this potentially reveals new information about the stopover ecology of western sandpipers. Radiotelemetry studies indicate that length of stay is only 1–3 d for adult western sandpipers refueling at Boundary Bay during the spring migration (Warnock

and Bishop 1998). Lank et al. (2003) estimated a somewhat longer length of stay for fall migrants, approximately 3–6 d for adults refueling at Sidney Island, a small site adjacent to Boundary Bay. If diet quality is typically lower during the adult's fall stopover period, then there could be a nutritional basis for the longer length of stay during fall migration.

Age-Related Comparisons among Fall Migrants

Adult and juvenile western sandpipers arrive at Boundary Bay after a 2,400-km flight across the Gulf of Alaska (Butler et al. 1996), with the adults preceding the juveniles by approximately 1 mo. Although there is little or no temporal overlap in their use of Boundary Bay (R. Will Stein, personal observation), adults ($n = 20$) and juveniles ($n = 12$) appear to consume the same limited array of invertebrates, with arthropod crustaceans again being the most prevalent (Seaman 2003). The later-migrating juveniles have a larger digestive system than the adults primarily because of an enlarged small intestine. An enlarged small intestine was already apparent in fully grown juveniles that had not yet initiated southward migration from Alaska (Stein 2002), and the magnitude of the age-related difference was nearly identical to that reported here for refueling migrants, approximately 15%. Although an overshooting of small intestine size is ubiquitous in growing birds (Starck 1998), fully grown juvenile western sandpipers apparently retain an enlarged small intestine that was initially associated with growth, during their first migration. Juvenile western sandpipers do not become adult-like in digestive system size until after they have settled on the wintering grounds, when the digestive system is markedly reduced in both age classes (Guglielmo and Williams 2003).

One of our primary objectives was to evaluate the functional significance of an enlarged digestive system to the later-migrating juveniles. A direct comparison with adults is complicated, however, because the juvenile's larger digestive system is not simply an enlarged version of the adult's. A reduced proventriculus and an enlarged small intestine suggest that juveniles may process prey differently than adults because digesta residence time is expected to be a function of organ size (Karasov and Hume 1997). Furthermore, the juvenile's larger digestive system was associated with lower total enzymic capacity due to lower total chitinase and lipase activities. Lower total chitinase activity in juveniles was due to a smaller proventriculus; as a consequence, the juveniles may be less efficient at extracting nutrients contained in prey with chitinaceous exoskeletons (Jackson et al. 1992). Lower extraction efficiency may influence the apparent lipid content of the diet, thereby accounting for their lower total lipase activity. Consistent with lower efficiency, fecal samples from juveniles contained more organic matter than those from adults. This suggests that the juveniles may process food less completely than the adults.

It is appealing to think that the later-migrating juveniles might have a different digestive strategy, that is, lower digestive efficiency and higher digesta passage rate, than adults, and this interpretation has some independent support. Juvenile dunlin (*Calidris alpina*) that were completing growth had relatively low digestive efficiency (57%; Norton 1970). Adult dunlin consuming the same diet are expected to have a substantially higher digestive efficiency (75%; Karasov 1990). However, studies of digestive performance in cold-stressed frugivorous passerine birds demonstrate that as the intake rate of food increases, passage rate increases and digestive efficiency does not change, at least in adults (McWilliams et al. 1999). If juvenile western sandpipers process food less thoroughly than adults, then juveniles may be able to compensate for lower efficiency by having higher intake and digesta passage rates, that is, by processing larger volumes of food less thoroughly. However, the potential benefits of maintaining an enlarged digestive system and increased volumetric and absorptive capacities may be offset by the system's high maintenance and running costs (Starck 1996, 1999).

Another possible explanation for the substantial age-related differences in digestive system structure and function is that diet quality may have been lower for the later-migrating juveniles. Although the juveniles consume the same types of invertebrates as the adults, they may be consuming a different age-class of these invertebrates, which has a different nutritional composition (e.g., Dobrzycka and Szaniawska 1993). The enlarged digestive system of juvenile western sandpipers may be adjusted to a different diet composition; however, the age-dependent difference in digestive system size is large and appears to have an ontogenetic origin (Stein 2002). Juvenile western sandpipers are confronted with additional energetic costs during their first migration that are associated with maintaining an enlarged digestive system when diet quality appears to be low; this might influence stopover duration and increase the duration of migration for juveniles, which would probably result in higher migration-related mortality for juveniles. Our results suggest that juvenile western sandpipers may process food differently from adults and/or have a lower-quality diet, and age-related differences in digestive system structure and function warrant further consideration in studies of migratory birds.

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